



FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2013/2014

Paulo Jorge Oliveira dos Santos

Effect of a swimming training session on the exhaled breath
temperature

março, 2014

FMUP

Paulo Jorge Oliveira dos Santos

Effect of a swimming training session on the exhaled breath
temperature

Mestrado Integrado em Medicina

Área: Imunologia

Trabalho efetuado sob a Orientação de:

Dra Mariana Couto

E sob a Coorientação de:

Professor Doutor André Moreira

Trabalho organizado de acordo com as normas da revista:

PEDIATRIC ALLERGY AND IMMUNOLOGY

março, 2014

FMUP

Eu, Paulo Jorge Oliveira dos Santos, abaixo assinado, nº mecanográfico 200802361, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 20/03/2014

Assinatura conforme cartão de identificação:

Paulo Jorge Oliveira dos Santos

NOME

Paulo Jorge Oliveira dos Santos

CARTÃO DE CIDADÃO OU PASSAPORTE (se estrangeiro)

E-MAIL

TELEFONE OU TELEMÓVEL

13512593

paulosanfc@gmail.com

912373671

NÚMERO DE ESTUDANTE

DATA DE CONCLUSÃO

200802361

20/03/2014

DESIGNAÇÃO DA ÁREA DO PROJECTO

Imunologia

TÍTULO DISSERTAÇÃO/MONOGRAFIA (riscar o que não interessa)

Effect of a swimming training session on the exhaled breath temperature

ORIENTADOR

Dra Mariana Couto

COORIENTADOR (se aplicável)

Professor Doutor André Moreira

É autorizada a reprodução integral desta Dissertação para efeitos de investigação e de divulgação pedagógica, em programas e projectos coordenados pela FMUP.

Faculdade de Medicina da Universidade do Porto, 20/03/2014

Assinatura conforme cartão de identificação: Paulo Jorge Oliveira dos Santos

Effect of a swimming training session on the exhaled breath temperature

Mariana Couto^{1,2,3}, Paulo Santos¹, Diana Silva^{1,4}, Luís Delgado^{1,3,4}, André Moreira^{1,3,4}

1- Immunology Lab, Faculty of Medicine, University of Porto

2- Imunoalergologia, Hospital e Instituto CUF Porto, Portugal

3- CINTESIS

4- Serviço de Imunoalergologia, Hospital São João EPE, Porto, Portugal

Running title: Exhaled breath temperature in elite swimmers

Correspondence to: Mariana Couto

Immunology Lab

Faculty of Medicine, University of Porto

Alameda Prof. Hernâni Monteiro

4200 – 319 Porto, Portugal

marianafercouth@gmail.com

00351 91 793 22 83

Fax: 00351 225 513 601

Conflict of interests: None

Funding source: None

Word count: 2273

Figures: 0

Tables: 2

Mariana Couto^{1,2,3}, Paulo Santos¹, Diana Silva^{2,4}, Carla Martins⁴, Luís Delgado^{2,3,4},
André Moreira^{2,3,4}

Effect of a swimming training session on the exhaled breath temperature

Pediatr Allergy Immunol

ABSTRACT

Background: By exercising, an inflammatory response of the airways occurs, with bronchial smooth muscle constriction and vasodilatation. The later could lead to an increase in exhaled breath temperature (EBT), possibly more pronounced in subjects with asthma. We aim to investigate the effect of a training session on EBT of elite swimmers and to assess if the impact is different in asthmatic vs non-asthmatic swimmers.

Methods: Swimmers that are annually screened for asthma in our Department were invited to this prospective study. The regular screening includes 2 visits in which subjects perform skin prick tests, spirometry before and after salbutamol inhalation and bronchial challenge with methacholine. Diagnosis of asthma was according to IOC-MC criteria. For those who agreed to participate, EBT was measured with X-halo thermometer before and after a training session. SPSS ($p < 0.05$) was used to compare baseline and post-training EBT; and Δ EBT between asthmatic and non-asthmatic swimmers.

Results: 22 swimmers accepted to participate, of which 10 had asthma. EBT significantly increased after the training session ($p = 0.020$). No significant differences were observed in mean Δ EBT among asthmatic and non-asthmatic swimmers ($p = 0.222$).

Conclusions: EBT increased after a training session. There was no difference in EBT between asthmatics and non-asthmatics.

Key words: asthmatics, exhaled breath temperature, swimmers, training

Correspondence to: Mariana Couto

Immunology Lab

Faculty of Medicine, University of Porto

57 Alameda Prof. Hernâni Monteiro
58 4200 – 319 Porto, Portugal
59 marianafercouth@gmail.com
60 00351 91 793 22 83
61 Fax: 0035122 551 36 01
62

63 INTRODUCTION

64 Asthma is defined as a clinical syndrome of intermittent respiratory symptoms triggered
65 by viral infections, environmental allergens, or other stimuli, and is characterized by
66 nonspecific airway hyperresponsiveness and inflammation (1). Vasodilatation is a
67 critical feature of inflammation, and angiogenesis and vascular remodeling are features
68 of chronic inflammatory diseases, such as asthma (2). The increased vascularity of the
69 airways in asthma is partly due to the elevated number of vessels associated with
70 angiogenesis (3) and partly due to vasodilation caused by the release of vasodilator
71 mediators, such as histamine, bradykinin and nitric oxide (NO).

72 As part of this inflammatory process, the increased vascularization of the airway
73 mucosa that occurs in asthmatics, leads to increased heat exchange during expiration
74 (4) and previous studies have reported that patients with asthma present an elevated
75 exhaled breath temperature (EBT) compared to healthy controls (5). The elevation of
76 exhaled NO as an inflammatory marker seen in asthmatics, has previously been shown
77 to correlate with increased EBT (5, 6), and therefore EBT has been proposed as a non-
78 invasive new biomarker for asthma control (7, 8).

79 Although the pathogenesis of the association of sports practice and airway injury is not
80 fully elucidated, some hypotheses have been proposed, one of which focuses upon
81 cooling of the airways caused by hyperpnea during exercise. Vigorous exercise
82 requires an increased ventilatory rate to meet higher muscular oxygen needs, which
83 results in the inhalation of a large volume of relatively cold and dry air and the loss of
84 heat from the respiratory mucosa. This mechanic noxious stimulus could cause
85 epithelial damage, and therefore the influx of inflammatory cells and their mediators
86 release (9-11). Also, higher levels of airway vascular permeability have been shown to
87 be a good predictor of the severity of EIA in asthmatics, which has led to the
88 microvascular theory of EIA based on functional abnormalities of endothelial cells in
89 newly generated microvessels in asthmatic airways (12).

90 It has been shown a significant increase in EBT in asthmatic children after exercise
91 (13), but this increase is not different between asthmatics and controls (14). In athletes
92 this was never investigated before and is a relevant issue as they present an increased
93 risk for asthma, especially those who take part in endurance sports, such as swimming
94 (10). We hypothesize that exercise would increase EBT, and a more pronounced
95 increase in would be seen in asthmatic athletes as a greater degree of response to
96 exercise as compared to healthy ones. Therefore, our aim was to investigate the effect

of a training session on EBT of elite swimmers, and to assess if the impact is different in asthmatic vs non-asthmatic swimmers.

METHODS

Study design and subjects

Elite swimmers of the FC Porto main swimming team who are annually screened for asthma and atopy at Allergy, Asthma and Sports Unit were invited to participate in this prospective study. The regular screening includes two visits, about 1 week apart, in which subjects are evaluated with skin prick tests, lung volumes before and after salbutamol inhalation, and airway responsiveness to methacholine. Swimmers are asked to withheld anti-asthmatic or anti-allergic medication that they might be taking for both visits, according to European Respiratory Society guidelines (15). Inhaled short acting β_2 -agonists were withheld for 8 hours before testing; inhaled long-acting β_2 -agonists, theophylline, and leukotriene antagonists were withheld for the last 72 hours; antihistamines were withheld for the last 7 days; and both inhaled and orally administered corticosteroids were withheld for the last month. At that time, they are diagnosed as having or not asthma, according to International Olympic Committee criteria (16). Later, for those who agreed to participate in the study, EBT was collected before and after a training session at their swimming pool.

In order to be eligible to participate in this study, a subject had to meet all the following criteria: elite level swimmer; free from respiratory infection in the last 3 weeks; provided signed and dated informed consent. A potential subject who met any of the following criteria was excluded from participation in this study: pregnancy; recent episode of hemoptysis; forced expiratory volume in the first second (FEV_1) lower than 60% of the predicted value or 1.5 L; neurological or psychiatric illness; lack of collaboration or coexistence of diseases that limit the patient's ability to carry out the tests; recent stroke or heart attack.

All subjects gave written informed consent, and the study was approved by ethical commission of Centro Hospitalar São João / Faculdade de Medicina da Universidade do Porto.

Procedures

Methacholine challenge

Non-specific bronchial hyperresponsiveness was measured by methacholine challenge, according to guidelines (15). Methacholine was delivered by inspiration triggered by an automatic dosimeter that delivers a single dose soon after the onset of a deep breath. The five-breath dosimeter method was used and the provocative dose causing a 20% fall in FEV₁ (PD₂₀) was determined.

Lung function

Spirometry was carried out according to the American Thoracic Society criteria (17). Lung function measurements were repeated 15 minutes after 400 µg of salbutamol in an aerochamber to assess reversibility.

Skin prick tests

Skin prick tests were carried out in accordance with international guidelines (18) with a standard battery of commercial extracts for common aeroallergens (Leti®, Madrid, Spain). Histamine dihydrochloride and diluent were used as positive and negative controls, respectively. Testing solutions were stored at +2 to +8°C when not in use. The largest and perpendicular diameter of the wheal for each of the allergens is measured and the following value calculated: largest + perpendicular diameter/2. A subject is defined as atopic in the presence of at least one positive result (regarded if the value calculated was ≥3 mm and controls showed adequate reactions) (18).

Exhaled breath temperature

EBT was measured using an X-halo device (Delmedica Investments, Singapore), 5 minutes before (baseline EBT) and 5 minutes after (post-exercise EBT) the swimming training session, according to previously validated methods (20). Briefly, the swimmers were requested to inhale freely through the nose and to exhale into the device at a rate and depth typical of their normal tidal breathing rhythm. The maneuver was continued until the built-in software of the instrument indicated that the measured value was stable. The decision of collecting EBT 5 minutes after the exercise was based on previous studies that have shown that this was the time point in which EBT reached the highest values, and decreases thereafter (14).

The time needed to achieve the stable EBT was recorded. Before and between measures, the device was kept at room temperature in order to maintain a stable starting temperature.

The swimmers performed their regular training session and no changes were imposed by the investigators. The intensity of the training session was recorded (categorized as 1: mild aerobic training; 2: moderate/intense aerobic training; 3: mild anaerobic training; and 4: moderate/intense anaerobic training) in order to identify a possible confounding effect. The training was performed at a chlorine disinfected open-air swimming-pool.

Body temperature

Oral temperature has been proposed to be related to the airways and/or the oral cavity, as both are part of the respiratory tract and are affected differently than systemic temperature during exercise (14), and therefore we have chosen axillary temperature to evaluate body temperature. It was measured with an axillary thermometer (MedCare®) before collecting baseline EBT.

Statistical analysis

Categorical variables are expressed as counts (%) and continuous variables as mean (standard deviation - SD) or, if not normally distributed, as median (interquartile range - IQR). Paired samples t-test was used to compare the differences between baseline and post-exercise EBTs. Differences between asthmatic and non-asthmatic swimmers were assessed with independent samples t-test for normally distributed data, Mann-Whitney for non-normally distributed data or Chi-Square for categorical variables. A new variable was created ($\Delta\text{EBT} = \text{post exercise EBT} - \text{baseline EBT}$), and used to assess if the variation of EBT was different among asthmatic and non-asthmatic subjects. Correlations were assessed with Spearman's test.

All analyses were performed using SPSS and STATA and considering a $p < 0.05$ for statistical significance.

RESULTS

Twenty-two elite swimmers accepted to participate, of which 10 had asthma. No differences were observed between asthmatic and non-asthmatic swimmers for demographic and personal characteristics, except for the expected lower PD_{20} among those with asthma (**table 1**).

189 **Table 1:**

190 ***Demographic and personal characteristics***

	Asthmatics (n=10)	Controls (n=12)	p
Men, n (%)	7 (70)	3 (23)	0.084
Age	17 ± 2.8	17 ± 2.9	0.946
Atopy, n (%)	4 (40)	4 (33)	1.000
PD20 methacholine	0.71 ± 0.6	4.39 ± 2.4	<0.001
FEV ₁ , Liters	4.26 ± 0.75	4.07 ± 0.92	0.622
FEV ₁ , % of predicted	111.10 ± 14.54	115.50 ± 8.89	0.393
FVC, Liters	5.13 ± 0.94	4.60 ± 1.20	0.271
FVC, % of predicted	114.50 ± 10.49	114.08 ± 11.48	0.931
FEV ₁ /FVC	83.52 ± 7.32	89.36 ± 7.18	0.074
FEF ₂₅₋₇₅ , Liters	4.22 ± 1.20	4.67 ± 1.17	0.387
FEF ₂₅₋₇₅ , % of predicted	97.3 ± 26.67	113.42 ± 22.54	0.140

191 Data presented as mean±SD, unless otherwise stated.

192 **FEF2575**: forced expiratory flow middle portion of FVC; **FEV1**: forced expiratory volume in one second;

193 **FVC**: forced vital capacity; **L**: liters; **PD20**: provocative dose inducing a 20% decrease in FEV1.

Exhaled breath temperature

Baseline and post-exercise EBT were not significantly different between asthmatic and healthy swimmers (**table 2**). EBT significantly increased after exercise in all subjects ($p=0.020$) (**figure 1**). This increase (Δ EBT) was not significantly different among asthmatic swimmers and the healthy ones ($p=0.222$) (**figure 2**). Correlation between PD_{20} and Δ EBT for asthmatic swimmers was not significant ($r=-0.103$, $p=0.777$). Because 6 (60%) asthmatic swimmers were under therapeutic with inhaled corticosteroids at the time of EBT collection, a second analysis was performed and no differences were observed between those with and without therapy ($p=0.853$).

Correlations between axillary temperature and both baseline and post-exercise EBTs were not significant ($p=0.972$ and $p=0.597$, respectively).

Regarding time to collect EBT, no differences were observed from baseline to post-exercise measurements in the global sample ($p=0.822$). Also, for baseline EBT collection, no differences occurred between asthmatic and non-asthmatic swimmers ($p=0.366$). However, for EBT collection after exercise, asthmatic swimmers took significantly less time to complete the measurement (4.2 ± 0.79 vs 5.0 ± 0.85 min, $p=0.035$).

Training

No significant differences were observed between asthmatic and controls regarding the intensity of the training session performed (**table 2**). The number of hours trained in the week of EBT collection was similar in the two groups (**table 2**).

215 **Table 2:**

216 ***Temperatures measurements and training evaluation***

	Asthmatic swimmers (n=10)	Healthy swimmers (n=12)	p
Baseline EBT, °C	34.08 ± 0.60	33.49 ± 0.95	0.102
Post-exercise EBT, °C	34.23 ± 0.55	33.94 ± 0.64	0.275
Body Temperature*	35.97 ± 0.42	36.02 ± 0.31	0.769
Hours of training in that week	9.4 ± 2.6	9.3 ± 3.4	0.910
Intensity of the session, <i>n</i>			0.121 [#]
Mild aerobic	8	3	
Moderate/severe aerobic	1	0	
Mild anaerobic	1	5	
Moderate/severe anaerobic	2	2	

218 Data presented as mean±SD, except otherwise stated. *Axillary temperature was used as a measure for
219 body temperature. [#]Chi-square test.

DISCUSSION

The hypothesis on the basis of this study was that elite swimmers would present increased EBT after a training session, due to the previously suggested idea that exercise would cause inflammation, and furthermore that asthmatic swimmers would experience a higher increase when compared to healthy controls. Interestingly, this could not be confirmed by this study. Although EBT was significantly increased in all subjects after training, supporting the hypothesis of heat loss during exercise, no differences were observed between swimmers with previously diagnosed asthma and healthy controls. It is therefore tempting to speculate that the theory of the heat loss as an ethiopathogenic mechanism of exercise-induced asthma lacks evidence.

Our study has some limitations. The sample size might under power the study to draw solid conclusions. Moreover, a cause that may have affected the results could be the 20 minutes of recovery (swimming with low effort) that swimmers made in the end of the training session and that decreases cardiac rhythm, respiratory frequency and the hyperpnea that makes EBT increase. If the temperature has been taken in the end of a more vigorous exercise instead at the end of the training this could lead to a marked results in Δ EBT.

Our results are consistent with previous studies that also have show an increase in EBT after exercise (13), but no differences between asthmatic subjects and healthy controls (14). That was different of other studies that shown differences in EBT of asthmatic patients compared to healthy controls (5). This raises the question whether the EBT could be a good inflammatory biomarker of asthmatics.

Previously has been hypothesized that dehydration of the airways with increased osmolality in the mucosal surface liquid leads to mast cell degranulation (21, 22), releasing bronchoconstriction mediators, such as bradykinin and histamine, and activating endothelial nitric oxide synthase (23), witch increase the vasodilation effect. This could suggest that elevated EBT could be a potential marker of a temporary effect on the airways, rather than of the long-term inflammation of the asthmatic disease. So the increase of EBT could be a physiological response of the airways to the increase of ventilatory rate, rather than a pathological pathway which can be involved in development of exercise-induced asthma.

This study leads us to hypotheses that the increase of EBT and there for the elevation of airway inflammation shown in previous studies (5, 6), during exercise (13), could be

253 a protect measure of the airways against heat loss and dehydration, that occurs with
254 the increase of ventilatory rate, rather than a pathological result.

255

256 **ACKNOWLEDGMENTS**

257 To Q-Pharma® for providing methacholine for bronchial provocation challenges. To
258 Carla Martins for her inestimable value and availability for performing bronchial
259 provocation challenges with methacholine. To swimmers who agreed to participate,
260 and to FCPorto staff for providing the logistic help which turned the project possible.

REFERENCES

1. Holgate S. Pathogenesis of asthma. *Clin Exp Allergy*. 2008;38:872-97.
2. Vignola AM, Mirabella F, Costanzo G, Di Giorgi R, Gjomarkaj M, Bellia V, et al. Airway remodeling in asthma. *Chest*. 2003;123:417S-22S.
3. Salvato G. Quantitative and morphological analysis of the vascular bed in bronchial biopsy specimens from asthmatic and non-asthmatic subjects. *Thorax*. 2001;56(12):902-6.
4. Paredi P, Kharitonov SA, Barnes PJ. Correlation of exhaled breath temperature with bronchial blood flow in asthma. *Respir Res*. 2005;6:15.
5. Piacentini GL, Peroni D, Crestani E, Zardini F, Bodini A, Costella S, et al. Exhaled air temperature in asthma: methods and relationship with markers of disease. *Clin Exp Allergy*. 2007;37(3):415-9.
6. Piacentini GL, Bodini A, Zerman L, Costella S, Zanolla L, Peroni DG, et al. Relationship between exhaled air temperature and exhaled nitric oxide in childhood asthma. *Eur Respir J*. 2002;20(1):108-11.
7. Melo RE, Popov TA, Solé D. Exhaled breath temperature, a new biomarker in asthma control: a pilot study. *J Bras Pneumol*. 2010;36(6):693-9.
8. García G, Bergna M, Uribe E, Yañez A, Soriano JB. Increased exhaled breath temperature in subjects with uncontrolled asthma. *Int J Tuberc Lung Dis*. 2013;17(7):969-72.
9. Schwartz LB, Delgado L, Craig T, Bonini S, Carlsen KH, Casale TB, et al. Exercise-induced hypersensitivity syndromes in recreational and competitive athletes: a PRACTALL consensus report (what the general practitioner should know about sports and allergy). *Allergy*. 2008;63(8):953-61.
10. Carlsen K-H, Anderson S, Bjermer L, Bonini S, Brusasco V, Canonica W, et al. Exercise-induced asthma, respiratory and allergic disorders in elite athletes: epidemiology, mechanisms and diagnosis: part I of the report from the Joint Task Force of the European Respiratory Society (ERS) and the European Academy of Allergy and Clinical Immunology (EAACI) in cooperation with GA2LEN. *Allergy*. 2008;63(4):387-403.
11. Couto M, Silva D, Delgado L, Moreira A. Exercise and airway injury in athletes. *Acta Med Port*. 2013;26(1):56-60.
12. Kanazawa H. VEGF, angiopoietin-1 and -2 in bronchial asthma: new molecular targets in airway angiogenesis and microvascular remodeling. *Recent Pat Inflamm Allergy Drug Discov*. 2007;1(1):1-8.
13. Peroni DG, Chinellato I, Piazza M, Zardini F, Bodini A, Olivieri F, et al. Exhaled breath temperature and exercise-induced bronchoconstriction in asthmatic children. *Pediatr Pulmonol*. 2012;47(3):240-4.
14. Svensson H, Nilsson D, Bjermer L, Tufvesson E. Exhaled breath temperature increases after exercise in asthmatics and controls. *Respiration*. 2012;84(4):283-90.
15. ERS Task Force on Standardization of Clinical Exercise Testing. Clinical exercise testing with reference to lung diseases: indications, standardization and interpretation strategies. *Eur Respir J*. 1997;10:2662-89.

16. Medical Commission of the International Olympic Committee. IOC's medical code. Lausanne: International Olympic Committee; 2002.
17. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26(2):319-38.
18. Heinzerling LM, Burbach GJ, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al. GA(2)LEN skin test study I: GA(2)LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. *Allergy*. 2009;64(10):1498-506.
19. Helenius I, Ryttilä P, Metso T, Haahtela T, Venge P, Tikkanen H. Respiratory symptoms, bronchial responsiveness, and cellular characteristics of induced sputum in elite swimmers. *Allergy*. 1998;53(4):346-52.
20. Popov TA, Dunev S, Kralimarkova TZ, Kraeva S, DuBuske LM. Evaluation of a simple, potentially individual device for exhaled breath temperature measurement. *Respir Med*. 2007;101:2044-50.
21. Brannan JD, Turton JA. The inflammatory basis of exercise-induced bronchoconstriction. *Phys Sportsmed* 2010; 38: 67-73.
22. Sheppard D, Eschenbacher WL. Respiratory water loss as a stimulus to exercise-induced bronchoconstriction. *J Allergy Clin Immunol* 1984; 73: 640-642.
23. Michel T, Vanhoutte PM. Cellular signaling and NO production. *Pflugers Arch* 2010; 459: 807-816.

Agradecimentos:

Agradeço à minha orientadora pela ajuda e preocupação durante todo o desenvolvimento do projeto. Agradeço também à minha família por me ajudar em termos anímicos durante todo o processo.

ANEXO

1 - Normas da revista: Pediatric Allergy and Immunology

Author Guidelines

PAI is only accepting manuscripts electronically via ScholarOne Manuscripts, an online submission site: <http://mc.manuscriptcentral.com/pai>

Complete instructions for preparing and submitting manuscripts online are provided at the submission site.

If you need assistance, please [phone](tel:14348172040) 1 434 817 2040 x 167 or e-mail Support@Scholarone.com.

Authors submitting a paper do so on the understanding that the work has not been published before, is not being considered for publication elsewhere and has been read and approved by all authors. All human and animal studies must be approved by an appropriate ethics committee or review board (depending on local arrangements), and a statement to this effect should be included in the methods section, or the reasons why it was not necessary if this is the case. All clinical investigations must have been conducted according to the principles expressed in the Declaration of Helsinki (<http://www.wma.net>).

The vested interests of authors (such as company affiliations or funding relevant to the study) must be declared.

Articles must be written in **correct scientific English** suitable for publication. Authors whose primary language is not English should obtain assistance with writing to avoid grammatical problems. Although articles are subject to review and editing, the journal does not hold itself responsible for all statements made by contributors.

Upon submission of a manuscript all co-authors should also be registered with **correct and updated e-mail addresses and academic titles**.

The work shall not be published elsewhere in any language without the written consent of the publisher.

The articles published in this journal are protected by copyright, which covers translation rights and the exclusive right to reproduce and distribute all of the articles printed in the journal. No material published in the journal may be stored on microfilm or videocassettes or in electronic databases and the like or reproduced photographically without the prior written permission of the publisher.

PAI employs a **plagiarism detection system**. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

After submission of a manuscript please address queries, if any, concerning the status of the manuscript to the editorial office –paieditorial@charite.de.

As the journal follows the Vancouver system for biomedical manuscripts, the author is referred to the publication of the International Committee of Medical Journal Editors: Uniform requirements for manuscripts submitted to biomedical journals. BMJ 1991;302: 338-41.



Line numbering

All texts submitted to PAI have to display line numbers (1, 2, 3, and so forth) in the left margin of the manuscript. (Line numbering can be added from the "Page Setup" or "Format" menu of word processing programs.) The line numbering should be continuous throughout the entire text. Start with the title page up to the final page. Do not [begin](#) numbering from 1 again at the top of each page.

ORIGINAL PAPERS

Information concerning the format of the manuscripts for all original papers:

Title page

The title page should contain the following information in the order given:

1. full title of manuscript, concise and informative, not exceeding 100 characters;
2. authors' full names;
3. authors' institutional affiliations including city and country;
4. a running title, not exceeding 40 characters and spaces;
5. the name, address and e-mail address of the author responsible for correspondence about the manuscript;
6. word count; number of tables and figures;
7. material in the electronic repository, if applicable.

Abstract page

A separate abstract page should contain the following:

1. authors' surnames and initials;
2. title of manuscript;
3. title of journal abbreviated as in reference list;
4. the word Abstract followed by a summary of the complete manuscript structured as follows (**max 250 words**):
 1. **background:** problem that prompted the study and aim(s) of the study
 2. **methods:** if the space is short., only the primary outcomes
 3. **results:** the most important findings only
 4. **conclusions:** the most important conclusion only
5. key words (max 10); listed in alphabetical order;
6. name and address of the author to whom requests for offprints should be sent.

Main text

The text is limited to:

- less than 2,500 words (not including abstract, figure legends and references)
- structured in introduction, methods, results and discussion
- general acknowledgments for consultations, statistical analyses, and the like should be listed at the end of the text,
- up to 30 references in the Journal's style (see below)
- up to 6 among figures (or panels of figures) and/or tables

If the manuscript is longer, reasons for increase in length, figure or table number or reference number should be stated in the cover letter. In general, the printed version of the manuscript should not occupy more than 6 pages.

References:

Number references consecutively in the order in which they are first mentioned in the text. List all authors when six or less; when seven or more, list first three and add et al. Identify references in text, tables, and legends by Arabic numerals (in parentheses). References (with the exception of review articles) must not exceed 30 in number. Use the style of the examples below which are based on the format used by US National Library of Medicine in Index Medicus. For abbreviations of journals, consult the List of Journals Indexed printed annually in the January issue of Index Medicus. Avoid using abstracts of articles as references. Unpublished observations, personal communications, and unaccepted papers may not be used as references, although references to written, not verbal, communications may be inserted (in parentheses) in the text.

Examples of correct forms of references are given below:

Journals: Chiba Y, Minagawa T, Mito K, et al. Effect of breast feeding on responses of systemic interferon and virus-specific lymphocyte transformation in infants with respiratory syncytial virus infection. *J Med Virol* 1987; 21: 7-14.

Books and monographs: Stiehm ER, Fulginiti VA. Immunologic disorders in infants and children. Philadelphia: WB Saunders 1973.

Chapter in book: Holt PG, Turner KJ. Regulation of IgE synthesis in man and experimental animals. In: Lessof MH, Lee TH, Kemeny DM, eds. Allergy, an international textbook. New York: John Wiley 1987: 69-87.

References in Articles – We recommend the use of a tool such as [EndNote](#) or [Reference Manager](#) for reference management and formatting. EndNote reference styles can be searched for here: <http://www.endnote.com/support/enstyles.asp>. Reference Manager reference styles can be searched for here: <http://www.refman.com/support/rmstyles.asp>

Figures

All graphs, drawings and photographs are considered figures and should be numbered in sequence with Arabic numerals and abbreviated Fig(s). Each figure should have a legend and all legends should be typed on a separate sheet and numbered correspondingly. Letters on figures should be in capitals. Figures should be planned to fit the proportions of the printed page. It is the policy of the journal for authors to pay for the full cost for the reproduction of their colour artwork. Therefore, please note that if there is colour artwork in your manuscript when it is accepted for publication, you are required to complete and return a **Colour Work Agreement form** before your paper can be published. This form can be downloaded online [here](#). Any article received at Wiley with colour work will not be published until the form has been returned.

Tables - Tables may be placed within the manuscript file and they should supplement, not duplicate, the information contained in the text. They should be on separate pages, one table per page, and should be numbered consecutively with Arabic numerals. Each table should be typed on a separate sheet, with due regard for the proportions of the printed page. A brief title should be provided directly above each table. Any abbreviations should be defined at the bottom of the table.

REVIEW ARTICLES: supplementary information

Text: less than 4,500 words not including abstract, figure legends and references (please supply a word count); up to 75 references in the Journal's style (if more, justification should be provided); figures and tables are important in review papers and up to 10 figures and/or tables (total) can be included in the text.

EDITORIALS: supplementary information

Text: less than 1,500 words not including abstract, figure legends and references (please supply a word count); up to 20 references in the Journal's style (if more, justification should be provided); up to 2 figures and/or tables (total) can be included in the text.

CORRESPONDENCE: supplementary information

LETTERS TO THE EDITOR

Letters to the Editor are short reports of clinical or biological observations without sufficient depth of investigation or number of cases to be classified as Original Articles. They may be subject to peer review. Letters are indexed in PubMed, accessible to literature searches, and cited like original articles.

(1) **SPACE:** Max. 3 pages in the printed issue, the average letter should have 2 pages, this means max. 2000 words, not including the figure legend(s) and references.

(2) **TITLE:** short and relevant, max 12 words

(3) **TITLE PAGE:** submit a complete title page including all authors affiliations

(4) **KEY-WORDS:** Provide a list of key words

- (5) SALUTATION: Start with the Salutation "To the Editor:"
- (6) SIGNATURE: Close with the author's name(s), academic degree(s), institutions(s), and location(s).
- (7) REFERENCES: max. 10 references;
- (8) FIGURES/TABLES: max. 3 figures and/or tables in the Journals style (at least 300 dpi)

CORRESPONDENCE and REPLIES

Correspondence concerning recent publications in the Journal will be considered for publication and accepted based on their scientific quality, and available space in the Journal. The authors of the referenced PAI article will be asked for a reply. Once approved by the Editor, the Correspondence and relevant Reply (if any) will both be published in the same issue.

- (1) SPACE: max. 1 page, this means max. 1000 words plus references and graphics (if any).
- (2) TITLE: short, relevant title, distinct from the title of the referenced article. Please note that all Replies should have the title "Reply to [Corresponding author's name]."
- (3) TITLE PAGE: complete title page
- (4) REFERENCES: List of references at the end of the letter; article being discussed as first reference. Number of references: max. seven. Replies should include the Correspondence to which they are replying as one of the references.
- (5) FIGURES/TABLES: Max. two graphic presentations (table or figure).
- (6) SALUTATION/ SIGNATURE: Begin with the salutation "To the Editor:" and close with the author's name(s), academic degree(s), institutions(s), and location(s).

REVISED MANUSCRIPTS

Revised manuscripts, if not differently indicated in the decision letter, must be returned within 3 months and must include the following items:

Responses to Comments that includes point-by-point responses to the comments made by the Reviewers, Editor, and Editorial Office for each of them numbered and labeled always as COMMENT and RESPONSE

Marked Manuscript Any text that was not part of the original manuscript but has now been added, underline formatting should be applied; to any text that was part of the original manuscript but has now been deleted, strikethrough formatting should be applied. Changes made on Figures and Tables should be clearly visible and provided as separate files labeled as 'Figure x Marked' and 'Table x Marked'. **Line numbering must be used** in the Marked Manuscript and numbers mentioned in the response to the comments.

Unmarked Manuscript The Unmarked Manuscript should be your revised manuscript just as you intend it for publication (if it is accepted). Line numbering need not be used in the Unmarked Manuscript too.

Supporting Information Pediatric Allergy and Immunology gives authors the opportunity to include data that would be inappropriate or impractical to include in the printed version. These data may substantially enhance the importance of the research and may also be of benefit to readers. All supporting information must be submitted as 'supplementary files for review' with the original manuscript via ScholarOne Manuscripts. 'Supporting Information' will be made available alongside the online version (only) of the published article (PDF). Please note that supporting information will not be copy-edited or typeset, but will be made available online in the exact form in which it is received and approved.

Author Services Online production tracking is now available for your article through Blackwell's Author Services. Author Services enables authors to track their article - once it has been accepted - through the

production process to online and print publication. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production so they don't need to contact the production editor to check on progress. Visit <http://authorservices.wiley.com/bauthor/> for more details on online production tracking and for a wealth of resources including FAQs and tips on article preparation, submission and more.

COPYRIGHT

If your paper is accepted, the author identified as the formal corresponding author for the paper will receive an email prompting them to login into Author Services; where via the Wiley Author Licensing Service (WALS) they will be able to complete the license agreement on behalf of all authors on the paper.

For authors signing the copyright transfer agreement

If the OnlineOpen option is not selected the corresponding author will be presented with the copyright transfer agreement (CTA) to sign. The terms and conditions of the CTA can be previewed in the samples associated with the Copyright FAQs below:

CTA Terms and Conditions http://authorservices.wiley.com/bauthor/faqs_copyright.asp

For authors choosing OnlineOpen

If the OnlineOpen option is selected the corresponding author will have a choice of the following Creative Commons License Open Access Agreements (OAA):

Creative Commons Attribution Non-Commercial License OAA

Creative Commons Attribution Non-Commercial -NoDerivs License OAA

To preview the terms and conditions of these open access agreements please visit the Copyright FAQs hosted on Wiley Author Services http://authorservices.wiley.com/bauthor/faqs_copyright.asp and visit <http://www.wileyopenaccess.com/details/content/12f25db4c87/Copyright--License.html>.

If you select the OnlineOpen option and your research is funded by The Wellcome Trust and members of the Research Councils UK (RCUK) you will be given the opportunity to publish your article under a CC-BY license supporting you in complying with Wellcome Trust and Research Councils UK requirements. For more information on this policy and the Journal's compliant self-archiving policy please visit: <http://www.wiley.com/go/funderstatement>.

For RCUK and Wellcome Trust authors click on the link below to preview the terms and conditions of this license:

[Creative Commons Attribution License OAA](#)

To preview the terms and conditions of these open access agreements please visit the Copyright FAQs hosted on Wiley Author Services http://authorservices.wiley.com/bauthor/faqs_copyright.asp and visit <http://www.wileyopenaccess.com/details/content/12f25db4c87/Copyright--License.html>.

Offprints Authors will receive a PDF offprint [free](#) of charge.

Pediatric Allergy and Immunology collaborates with Wiley's open access journal *Immunity, Inflammation and Disease* to enable rapid publication of good quality research that we are unable to accept for publication in *Pediatric Allergy and Immunology*. Authors will be offered the option of having the paper, along with any related peer reviews, automatically transferred for consideration by the Editor of *Immunity, Inflammation and Disease*. Authors will not need to reformat or rewrite their manuscript at this stage, and publication decisions will be made a short time after the transfer takes place. The Editor of *Immunity, Inflammation and Disease* will accept submissions that report well-conducted research which reaches the standard acceptable for publication. *Immunity, Inflammation and Disease* is a Wiley Open Access journal and article publication fees apply. For more information please go to www.immunityinflammationdisease.com.